Nutrient removal from UASB effluent in agro-industries

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Abstract
Phosphorus and nitrogen are important elements, making a major contribution to agricultural and industrial development, but their release to natural water bodies are the main causes of eutrophication. Anaerobic digestion yields effluents rich in ammonium and phosphate and poor in biodegradable organic carbon, thereby making them less suitable for conventional biological nitrogen and phosphorus removal. In addition, the demand for fertilizers is increasing, energy prices are rising and global phosphate reserves are declining. This requires both changes in wastewater treatment technologies and implementation of new processes. In this contribution the combination of an ureolytic MAP (magnesium ammonium phosphate) precipitation and autotrophic nitrogen removal is described on the anaerobic effluent of a potato processing company to obtain a more sustainable and cheaper method than conventional wastewater treatment processes. The results obtained during this experiment (6 weeks period) show that it is possible to recover phosphate as struvite and remove nitrogen with the autotrophic nitrogen process from wastewater after anaerobic digestion coming from a potato processing company. However further research is necessary to obtain stable results during several months, especially for the nitrite:ammonium ratio produced by the partial nitritation reactor.

Keywords: Autotrophic nitrogen removal; nutrient recovery; struvite.

INTRODUCTION

Nowadays, anaerobic digestion plants are being developed that minimize energy consumption, CO_2 emission and sludge production. However, these systems typically yield effluents rich in ammonium and phosphate and poor in biodegradable organic carbon, thereby making them less suitable for conventional biological nitrogen and phosphorus removal. Readily biodegradable organic matter needs to be bypassed towards aerobic post-treatment in order to achieve the final nutrient effluent standards, thus reducing the potential biogas yield and increasing the waste sludge production.

Global phosphate reserves are declining and occur exclusively as phosphate ore. Through an increasing reliance of many industries on phosphate, there is a growing necessity for sustainable phosphate management. Phosphate recovery by struvite or magnesium ammonium phosphate (MAP) is becoming an important issue because it permits its recycling in the fertilizer industry due to its valuable as a slow release fertilizer. Ureolytic phosphate precipitation (UPP) is an alternative for phosphate removal (Meesschaert *et al.*, 2007; Carballa *et al.*, 2008; Desmidt *et al.*, 2009). During this process an alternative way for increasing the pH is used. Instead of NaOH, the pH was increased by means of bacterial urease activity. The enzyme urease hydrolyses urea, which is added to anaerobic sludge, to ammonia and carbamate. The latter compound decomposes spontaneously to carbonic acid and a second molecule of ammonia (Mobley *et al.*, 1989). These products subsequently equilibrate in water to form bicarbonate and 2 moles of ammonium The overall reaction can be written as follows:

$$CO(NH_2)_2 + H^+ + 2\ H_2O \rightarrow 2\ NH_4^+ + HCO_3^- \quad (1)$$

Due to the ammonia release, pH increases during ureolysis. The process thus introduces extra ammonium in the system.

The autotrophic nitrogen removal (ANR) process, as alternative for the conventional nitrification-denitrification process, consumes 63% less oxygen and 100% less biodegradable organic carbon compared to the conventional process and therefore has a lower operating cost (Verstraete and Philips, 1998). This process consist of a combination between partial nitritation and the anaerobic ammonium oxidation or Anammox process. Anammox bacteria are able to consume ammonium and nitrite under anaerobic conditions according to the reaction (Strous *et al.*, 1998):

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (2)$$

Ammonium is oxidized to nitrogen gas using nitrite as the electron acceptor. A small fraction of nitrite is anaerobically oxidized to nitrate and yields electrons for the oxidation of cell material for cell growth. Hence, the molar ratio of ammonium and nitrite in Anammox catabolism is 1:1,32. In view of coupling partial nitritation with Anammox, nitrite oxidizing activity should be suppressed and ammonium should only be oxidized for about 50% to nitrite. Different influencing factors can be used to engineer a system that accomplishes this requirement. The most important environmental parameters to obtain partial nitritation are the free ammonia (FA, NH_3) and free nitrous acid (FNA, HNO_2) concentration, the temperature, hydraulic retention time, pH and dissolved oxygen concentration (Van Hulle *et al.*, 2010).
The combination of anaerobic digestion, phosphate recovery by precipitation as struvite and nitrogen removal with the ANR process could be a more sustainable and cheaper method than conventional wastewater treatment processes without recuperation of the nutrients.
In this contribution we investigate the combination of UPP and ANR to remove phosphate and both the endogenous ammonium and the ammonium derived from the ureolysis in the effluent of an UASB (Upflow Anaerobic Sludge Blanket) of a potato processing company.

MATERIALS AND METHODS

Experimental set-up

Ureolytic phosphate precipitation

The ureolytic phosphate precipitation was tested at lab-scale on anaerobic effluent of a potato processing company (Agristo NV, Harelbeke, Belgium). Before the wastewater was fed to the crystallization reactor, where struvite precipitation occurred, air stripping was applied to increase the pH. Urea (1.5 %) was dosed to anaerobic sludge in an urease breeder (figure 1), with a working volume of 1 L. The hydraulic retention time of the sludge was 2 days. Due to bacterial urease activity, the overflow to the crystallization reactor (built according to BNB EN ISO 11733; working volume of 3.7 L) resulted in an increase in pH and ammonium concentration. The hydraulic retention time of the wastewater in the crystallization reactor varied between 6 and 7 hours. The addition of $MgCl_2.6H_2O$ (2 %) to the reactor and the presence of the ammonium derived from the ureolysis and both ammonium and phosphate in the wastewater resulted in the precipitation of struvite according to the following reaction:

$$Mg^{2+} + NH_4^+ + HPO_4^{2-} + 6H_2O \rightarrow MgNH_4PO_4.6H_2O + 2H^+ \quad (3)$$

The effluent from the crystallization reactor was directed to a decanter, where separation between the final effluent and the sludge occurred.

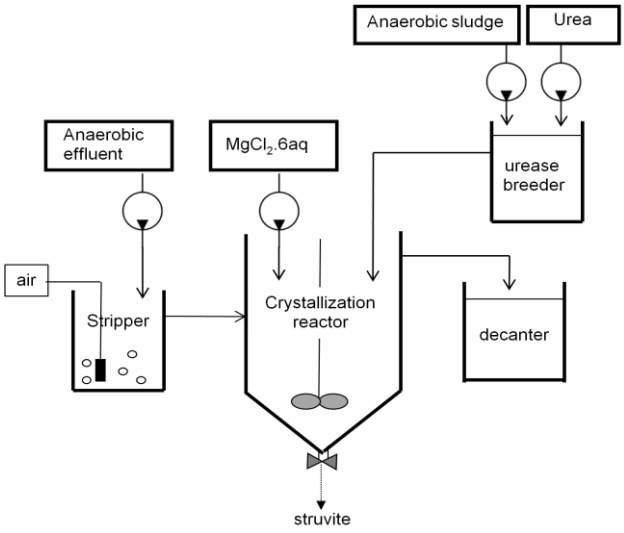

Figure 1. Schematic overview of the ureolytic phosphate precipitation

Partial nitritation reactor

After decantation, the effluent of the ureolytic phosphate precipitation was fed to a continuous reactor of 20 L, inoculated with nitrifying sludge of a potato processing company (Agristo NV, Harelbeke, Belgium).

According to Volcke *et al.* (2007) the dilution rate depends on the influent ammonium concentration and the pH. For an influent ammonium concentration of 150 mg.L^{-1} and a pH of 8, the range of dilution rates that guarantee stable nitrite formation is 0.5 - 1.25 d^{-1}. So, a HRT of 1.4 days is a good choice to ensure robust operation. Volcke *et al.* (2002) also found that the obtained TNO_2:TAN (total nitrite nitrogen:total ammonium nitrogen) ratio in the effluent is highly influenced by the buffer capacity of the influent, that varies with influent pH and TIC concentration. In this way, the latter seem suitable for controlling the TNO_2:TAN ratio. Because the ratio TIC:TAN (total inorganic carbon: total ammonium nitrogen) in the influent varies between 1.4 and 1.7, continuous aeration of the reactor would result in almost a complete conversion of ammonium to nitrite, the DO in the reactor was controlled to obtain only a 50% conversion of ammonium. According to Ruiz et. al (2003) an ammonium and nitrite accumulation was observed when the DO was kept below 0.5 mg. L^{-1}.

The operational conditions during the partial nitritation process were: no sludge retention, about 1.4 days of HRT, a temperature of 35° C and a pH of 8-8.3. The DO was controlled between 0.1 and 0.5. No pH adjustment occurred in the reactor.

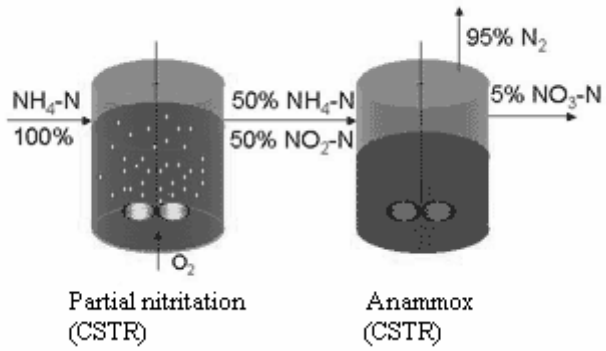

Figure 2. Simplified scheme of the autotrophic nitrogen removal

Anammox reactor

The Anammox bacteria were enriched from conventional sludge of a vegetable processing company (Unifrost NV, Koolskamp, Belgium) in a continuous lab-scale reactor with a volume of 3,7 L, filled with carrier material which consisted of poly-urethane. In the middle of the carrier material a glass tube was provided, making mixing and feeding at the bottom of the reactor possible. The effluent was drained off at the upper side of the reactor. The reactor was continuously mixed with a mechanical stirrer and kept at a temperature of 35 °C. To enrich the Anammox bacteria the reactor was first fed with synthetic medium described in table 1. The media was fed to the reactor with a hydraulic retention time (HRT) of 40 hours. After 3 months of operation with synthetic wastewater, the Anammox reactor was fed with the effluent of the partial nitritation reactor. Due to the slow growth rate of the Anammox bacteria, only a part (1/6) of the effluent of the partial nitritation was fed to the Anammox reactor. An upscale of the reactor to a volume of 20 L or a higher flow rate would be necessary to treat all the effluent of the partial nitritation. Figure 2 shows a simplified scheme of the autotrophic nitrogen removal, which is a combination of the partial nitritation reactor and the Anammox reactor.

Table 1. Composition of the synthetic medium used during the start-up of the Anammox reactor

Synthetic wastewater	**CSTR ($g.L^{-1}$)**
$KHCO_3$	1.25
KH_2PO_4	0.025
$CaCl_2.6H_2O$	0.45
$MgSO_4.7H_2O$	0.2
$(NH_4)_2SO_4$	0.24-0.48
$NaNO_2$	0.25-0.50
$FeSO_4.7H_2O$	5
EDTA	5
Trace elements solution*	1 $ml.L^{-1}$

* Described by Van de Graaf *et al.* (1996)

Analytical methods

The pH was measured with a pH meter (Mettler Toledo seven multi). Dissolved oxygen was measured with an oxygen meter Oxi 315 (WTW). Ammonium and magnesium were determined with a Dionex DX-100 chromatograph equipped with a conductivity detector. Phosphate, nitrite and nitrate were measured with a Dionex series 4500i ion chromatograph equipped with a conductivity detector. The dry matter content and its mineral fraction were determined according to standard methods (Greenberg *et al.*, 1992). Inorganic carbon (IC) was analyzed by a Shimadzu total carbon analyzer TOC-V_{CPN}. The crystals formed in the ureolytic phosphate reactor were examined and identified by XRD, using a Stoe Stadi P unit with Cu K_α radiation at 0.1541 nm with an image plate as detector. The DO in the partial nitritation reactor was controlled by means of a LDO electrode coupled with a SC-100 controller of Hach-Lange.

Fluorescent in-situ hybridization (FISH)

The biomass was fixed in a 4% paraformaldehyde solution. FISH was performed according to Amann et al (1990). The probes used in this study were Nso1225 labeled with fluorescein for b-proteobacterial AOB (Mobarry *et al.* 1996) combined with Amx820 labeled with Cy3 for the anammox bacteria "*Candidatus Brocadia*" and "*Candidatus Kuenenia*" (Schmid *et al.* 2000). Image acquisition was done on a Zeiss Axioskop 2 Plus epifluorescence microscope.

RESULTS AND DISCUSSION

Ureolytic phosphate precipitation

Table 2 shows the characteristics of the influent and effluent of the ureolytic phosphate precipitation. The hydrolysis of urea resulted in a pH increase and an increase in ammonium and inorganic carbon concentration. Also the addition of magnesium chloride to the reactor resulted in a higher magnesium concentration in the effluent. During the experiments a high phosphate removal efficiency of 83 ± 1 % was observed, resulting in a final effluent concentration of 10 ± 2 mg.L^{-1} PO_4-P (Figure 3). The crystals in the reactor were identified as struvite by XRD.

The effluent of the ureolytic phosphate precipitation is fed to the partial nitritation after decantation.

Table 2. Characteristics of the influent of the ureolytic phosphate precipitation

Parameter	Influent UPP*	Effluent UPP*
pH	7.9 ± 0.2	8.3 ± 0.1
PO_4-P (mg.L^{-1})	53 ± 7	10 ± 2
NH_4-N (mg.L^{-1})	140 ± 8	174 ± 16
Mg^{2+} (mg.L^{-1})	37 ± 10	157 ± 27
IC* (mg.L^{-1})	254 ± 12	281 ± 47

*IC = Inorganic carbon

*UPP = Ureolytic phosphate precipitation

Autotrophic nitrogen removal

After phosphate precipitation the ammonium was removed by a combination of a partial nitritation and Anammox process. As mentioned before the TIC:TAN in the influent of the partial nitritation varied between 1.4 and 1.7. Because the pH of the influent is around 8.3 and the TIC:TAN ratio is higher than one, the DO in reactor was controlled between 0.1 and 0.5 mg.L^{-1}. Continuous aeration with a DO higher than 0.5 resulted in almost a complete conversion of ammonium to nitrite (results not shown). During the first three weeks of operation (5 samples) the nitrogen removal efficiency was 85 ± 5 % and resulted in a final effluent concentration of 27 ± 10 mg.L^{-1} NH_4-N (Figure 3).

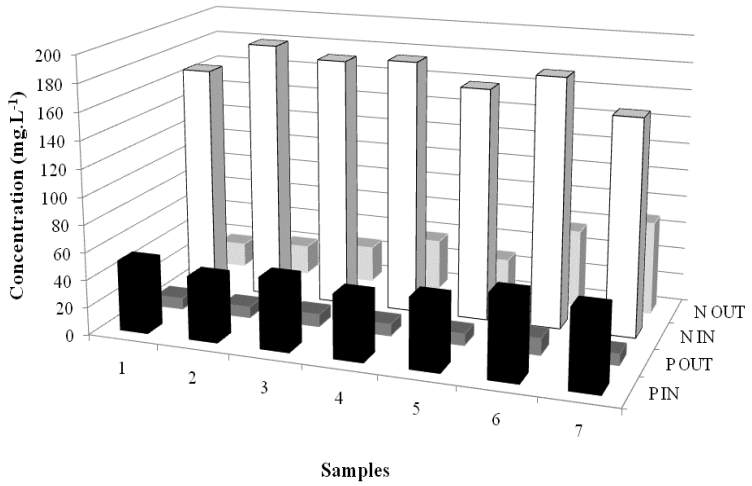

Figure 3. Phosphorus and nitrogen concentrations in the influent and effluent of respectively the ureolytic phosphate precipitation and autotrophic nitrogen removal process

In the following weeks the removal efficiency decreased to respectively 70 % for sample 6 and 60 % for sample 7. In general, Anammox removes only 90% of the incoming nitrogen as ammonium/nitrite and leaves 10% of nitrogen as nitrate in the effluent (Kumar and Lin, 2010). This was also observed during our experiments as 18 ± 4 $mg.L^{-1}$ NO_3-N was present in the effluent, starting from an influent concentration of 174 ± 16 $mg.L^{-1}$ NH_4-N. The decrease in removal efficiency was probably because the nitrite:ammonium ratio in the effluent of the partial nitritation was 1.3 ± 0.4. This variation resulted in an build up of the nitrite concentration in the effluent of the Anammox reactor. However another 6 weeks of operation (results not shown) under the same circumstances resulted in a removal of 70 % of the ammonium but only 15 % of the nitrite. From this point on the reactor was fed with the effluent of the ureolytic phosphate precipitation instead of the effluent of the partial nitritation. This means that the autotrophic nitrogen removal was operated in one reactor instead of two. By feeding the Anammox reactor with the effluent of the partial nitritation (without sludge retention), some sludge of the partial nitriation was caught in the carrier material of the Anammox reactor leading to a co-culture of micro-organisms. The presence of anammox bacteria in the Anammox reactor for the first period (operation in two reactors) was confirmed by FISH analysis. Research is ongoing for the autotrophic nitrogen removal in one reactor.

CONCLUSIONS

The results obtained during this experiment (6 weeks period) show that it is possible to recover phosphate as struvite and remove nitrogen with the autotrophic nitrogen process from wastewater after anaerobic digestion coming from a potato processing company. However further research is necessary to obtain stable results during several months, especially for the nitrite:ammonium ratio produced by the partial nitritation reactor. Controlling the nitrite:ammonium ratio is essential to avoid toxic nitrite concentrations, which inhibit the Anammox conversion. When the nitrite:ammonium ratio in the Anammox feed deviates from the ideal ratio, its conversion efficiency will decrease. There are several control strategies to obtain an optimal of nitrite:ammonium ratio such as controlling the pH and/or the DO in the partial nitritation reactor. In this study, controlling the DO in the reactor was not sufficient to obtain a stable nitrite:ammonium ratio in the effluent of the partial nitritation. Another approach is the operation of the autotrophic nitrogen removal in one reactor (OLAND) instead of two reactors (partial nitritation and Anammox).

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